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Properties and intracellular modulating factors of hyperpolarization-activated cation currents in rat dorsal root ganglion neurones

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1. Currents activated by membrane hyperpolarization were measured in rat dorsal root ganglion (DRG) neurones voltage-clamped by a conventional whole-cell or nystatin-perforated configuration to investigate their properties and to explore intracellular modulating factors.
2. Under both the nystatin-perforated and the conventional whole-cell condition using a  $\text{Cl}^-$ -rich intracellular solution, the resting membrane potential measured by a current clamp configuration in rat DRG neurones was  $-48.9 \pm 2.5$  mV and  $-55.9 \pm 0.8$  mV, respectively. They were significantly different from each other.
3. In the voltage-clamped cells by the conventional whole-cell configuration, hyperpolarizing step pulses from a holding potential of -60 mV to that between -70 and -130 mV elicited slowly activating inward currents. The current-voltage relationship for these currents showed an obvious inward rectification.
4. The density of the hyperpolarization-activated current in each cell was plotted against membrane capacitance ( $n=69$ ). The current density was not influenced by the cell size.
5. Voltage dependence of the hyperpolarization-activated current was investigated. This current began to be activated at -70 mV and almost fully activated at -130 mV. A half-maximal activation potential ( $V_{half}$ ) was  $-86.3 \pm 6.4$  mV and a slope factor was  $9.2 \pm 1.1$  (n = 7).
6. A reversal potential of hyperpolarization-activated current was  $-20.3 \pm 5.0$  mV (n = 7).
7. The  $V_{half}$ , slope factor and current density of the hyperpolarization-activated current obtained with a low  $\text{Cl}^-$  intracellular solution were not significantly different from those with the  $\text{Cl}^-$ -rich intracellular solution.
8. Total replacement of the extracellular  $\text{Na}^+$  by N-methyl-(D)-glucamine ( $\text{NMDG}^+$ ) reduced the hyperpolarization-activated current by  $25.6 \pm 4.9\%$  (n = 8). Total replacement of the extracellular  $\text{K}^+$  and both  $\text{Na}^+$  and  $\text{K}^+$  by  $\text{NMDG}^+$  almost abolished the hyperpolarization-activated current. The degrees of reduction were  $75.14 \pm 5.0\%$  (n = 6) and  $89.5 \pm 2.2\%$  (n = 3), respectively.
9. Extracellular applications of 2 mM  $\text{Cs}^+$ , 2 mM  $\text{Ba}^{2+}$  and 20 mM tetraethylammonium (TEA) inhibited the amplitude of the hyperpolarization-activated current by  $79.2 \pm 6.4\%$  (n = 6),  $34.5 \pm 7.1\%$  (n = 8) and  $19.2 \pm 8.6\%$  (n = 7), respectively. However this current was insensitive to 1 mM 4-aminopyridine (4-AP).
10. cAMP (1 mM), cGMP (1 mM) and ATP at 2 mM but not 1 mM added to the intracellular solution shifted  $V_{half}$  by 15-20 mV toward more positive potentials.
11. In the nystatin-perforated configuration, the  $V_{half}$  of the hyperpolarization-activated current was approximately 8.5 mV more posi-

tive than that obtained under conventional whole-cell voltage clamp condition with the low  $\text{Cl}^-$  intracellular solution. After measurement of  $V_{half}$  with nystatin-perforated configuration, the patch membrane rupture made the same cell to be under conventional whole-cell voltage clamp condition. This protocol shifted  $V_{half}$  by about 15 mV toward more negative potential.

12. These properties of the hyperpolarization-activated current ( $I_h$ ) in rat DRG neurones in the present study almost agree with those in other neuronal cells.  $I_h$  in rat DRG neurones was found to be regulated by not only cytosolic cAMP but also cGMP and ATP.  $I_h$  channel may contribute towards setting the resting membrane potential in rat DRG neurones.

### Effect of leptin on catecholamine release from rat chromaffin cells and pheochromocytoma cells.

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To investigate the effects of leptin on catecholamine release from rat adrenal medullary chromaffin cells and rat pheochromocytoma cells (PC12), I performed RT-PCR to detect mRNA of leptin receptors (Ob-R) and Western blot to detect phosphorylation of MAP kinase which occurred when Ob-R was activated, and measured catecholamine release. The following results were obtained:

1. The mRNA of the long form leptin receptor (Ob-Rb) which had high ability of signal transduction was present in rat chromaffin cells and PC12.
2. Leptin caused phosphorylation of MAP kinase in both rat chromaffin cells and PC12.
3. Leptin had no acute effect on the spontaneous dopamine release in PC12. However, leptin added chronically to the culture medium for more than 4 days inhibited spontaneous dopamine release from PC12 differentiated by NGF, but not from undifferentiated cells.

4. Leptin had no effect on the dopamine release response to 100  $\mu\text{M}$  ACh, 60 mM KCl and 100  $\mu\text{M}$  ATP in NGF-differentiated PC12. Leptin also had no effect on the dose dependency of dopamine release response to ATP in NGF-differentiated PC12. Thus, the inhibition of calcium channels seems not to contribute to the inhibitory effect of leptin on spontaneous dopamine release from NGF-differentiated PC12.

5. Leptin increased the ratio of noradrenaline to dopamine in NGF-differentiated PC12.
6. Leptin had no acute effect on spontaneous and ACh-induced catecholamine release from rat chromaffin cells.

From results mentioned above, it was suggested that a functionally active Ob-R was present in rat chromaffin cells and PC12. Leptin had no effect on the catecholamine release response in rat chromaffin cells. Leptin chronically inhibited spontaneous dopamine release from PC12 differentiated by NGF. Fi-